Protein N-Myristoylation as a Chemotherapeutic Target for Cancer

Ronald L. Felsted, Constance J. Glover, Kathleen Hartman*

The lipid modification of proteins by N-myristoylation refers to the transfer of myristic acid (C14:0) from myristoyl-coenzyme A (CoA) in amide linkage to the NH₂-terminal glycine residues of a number of viral and eukaryotic cellular proteins by N-myristoyl-CoA:protein N-myristoyltransferase (NMT) (1-3). For many proteins synthesized on free polyribosomes, cotranslational N-myristoylation is required for subcellular targeting, protein conformation, and/or biological activity.

NMT is a ubiquitously distributed eukaryotic enzyme accounting for the N-myristoylation of a substantial number of proteins, some of which are required for signal transduction and regulatory functions important in cellular growth control (e.g., serine/threonine/tyrosine kinases, protein phosphatases, and G-proteins). Thus, NMT appears to be a critical cellular enzyme, the disruption of which has been shown to be lethal in the yeast Saccharomyces cerevisiae (4) and in the fungi Cryptococcus neoformans and Candida albicans (5,6). Considering the cumulative genetic mutations involved in carcinogenesis (7), including changes in oncogenes that could result in altered expression and/or function(s) of N-myristoylated oncoproteins (8), it is also likely that NMT is functional in aberrant cellular processes that account for progression to the malignant cell phenotype. To the extent that NMT and/or N-myristoylated proteins are found to be involved in carcinogenesis, protein N-myristoylation should be examined as a possible chemotherapeutic target for cancer (2).

However, progress in furthering this goal will require 1) establishing an N-myristoylation causal and/or dependency relationship with cancer, 2) an understanding of the mechanism(s) regulating protein N-myristoylation in mammalian cells, and 3) developing approaches to selectively interfere with critical N-myristoylation steps while avoiding general toxicity.

The comparison of enzymes (normal versus tumor cell) from diverse metabolic pathways has revealed quantitative changes in activity that may confer a selective growth advantage to malignant cells (9). Certain of these enzymes have been considered as diagnostic/therapeutic targets for cancer. The report by Magnuson et al. (10) in this issue of the Journal is the first comparison of NMT activity in tumors (average increase, fivefold) relative to normal tissue. The extensive analysis of the well-characterized azoxymethane-induced rat colon cancer model provides a convincing correlation between levels of NMT activity and tumor progression. A smaller increase (average increase, twofold) in three human colon adenocarcinomas suggests that a similar relationship may extend to humans.

The tyrosine kinase activities of the N-myristoylated pp60⁶⁰⁰-src and pp62⁶⁰⁰-yes protein tyrosine kinases are also significantly elevated in colon carcinoma cell lines and primary colon cancers relative to normal cells (8,11,12). It appears that the heightened pp60⁶⁰⁰-src activity in colon cancer correlates with both an increase in enzyme synthesis and an association of the activated kinase with the cytoskeleton. Magnuson et al. (10) suggest that the increased NMT activity in colon cancer may be required to support a parallel increase in the expression of N-myristoylated proteins (i.e., pp60⁶⁰⁰-src and pp62⁶⁰⁰-yes) having regulatory roles in colonic neoplasms. Since a relationship has been previously established between elevated pp60⁶⁰⁰-src activity, cellular transformation, and an N-myristoylation-dependent association of both cellular and viral pp60⁶⁰⁰ with the cytoskeleton (and/or cell membrane) (13,14), it is possible that the increased synthesis of pp60⁶⁰⁰-src in colon cancer requires an increased level of cotranslational N-myristoylation to facilitate the N-myristoylation-dependent targeting of newly synthesized pp60⁶⁰⁰-src to the cytoskeleton.

The protein N-myristoylation pathway is a complex process involving the carefully coordinated participation of several different enzymes (e.g., methionylaminopeptidase, fatty acid synthetase, and long-chain acyl-CoA synthetase), the access of NMT to pools of myristoyl-CoA, and the timely N-myristoylation of nascent proteins to avoid potentially interfering reactions (e.g., N-acetylation and polypeptide folding) (15,16). In vitro kinetic studies and in vivo genetic analyses of protein N-myristoylation in S. cerevisiae suggest that the availability of myristoyl-CoA pools is critical and is maintained by both de novo fatty acid synthesis and activation of imported myristate. However, differences in the regulation of myristoyl-CoA synthesis in yeast and C. neoformans are known (5), and one must assume that further differences will be found in mammalian cells. When one considers that much of the information on protein N-myristoylation...
tion is based on the \textit{S. cerevisiae} model, then assessing protein \textit{N}-myristoylation as a chemotherapeutic target for cancer will require a much more extensive characterization of the \textit{N}-myristoylation phenomenon in mammalian cells.

Recent studies (1,7-19) reveal that mammalian NMT exhibits an appreciable level of complexity with notable differences from the yeast enzyme. Mammalian NMTs with molecular masses from 48 to 390 kd and with subunits of 48 to 67 kd have been reported, suggesting that multisubunit forms of the enzyme exist in mammalian cells. In contrast, yeast NMT is a monomer of approximately 55 kd. In vitro studies on bovine brain NMT have identified at least two interconvertible forms of the enzyme that result from multimerization of approximately 60 kd NMT subunit(s) and/or complex formation with other cellular proteins (17-19). A fully active NMT monomer of 48-50 kd that arises by proteolytic removal of the 12 kd \textit{N}-terminus of the 60 kd subunit was also found. The removal of this domain with no effect on enzyme activity is consistent with the possibility that the \textit{N}-terminus of NMTs has noncatalytic functions, such as the targeting of the enzyme to ribosomal complexes and/or regulating interactions of the enzyme with proteins controlling substrate availability. Also, the ability of NMT substrate (i.e., myristoyl-CoA) and products (i.e., \textit{N}-myristoylpeptides) to promote the reversible dissociation of the larger NMT multimers into smaller enzyme forms in vitro is suggestive of a mechanism that could be instrumental in regulating NMT activity in vivo. While such speculations remain to be established, an appreciation of the mechanism(s) regulating the mammalian NMT should reveal opportunities for modulating enzyme activity.

The considerable progress in advancing protein \textit{N}-myristoylation as a chemotherapeutic target toward infectious pathogens contributes pharmacologic insights for applying prospective anti-\textit{N}-myristoylation agents to cancer. The rational design of altered NMT substrates or substrate precursors provides a direct approach for modulating \textit{N}-myristoylprotein synthesis or function. One such strategy involves the development of a series of heteroatom analogues of myristate containing single oxygen or sulfur substitutions for methylene groups that are readily taken up by cells, activated by a cellular acyl-CoA synthetase, and incorporated into a limited subset of normally \textit{N}-myristoylated proteins by NMT (20). This selective substitution plus the resulting changes in the subcellular membrane and cytosolic distributions of only a few of the resulting heteroatom \textit{N}-acylproteins most likely accounts for the relative nontoxicity of this approach. Heteroatom-myristate analogues have been shown to inhibit human immunodeficiency virus-1 replication by incorporation into the virus polyprotein \textit{p55}^{pr}, thus interfering with its \textit{myristate}-dependent membrane targeting and maturation-dependent proteolytic processing (21). Similarly, heteroatom \textit{N}-acylation interferes with the membrane association of the Rous sarcoma virus v-tyrosine kinase \textit{p60}^{src} (22), an association required for the virus’s transforming activity. To the extent that \textit{N}-myristoylation is involved in the cytoskeletal association of pp60 \textit{c-src} and pp62 \textit{yes} in colon cancer, then it also may be possible to use the heteroatom myristate analogues to block kinase subcellular targeting and thereby prevent the phosphorylation of cytoskeleton and membrane protein(s) that contribute to the malignant phenotype.

An alternate strategy of targeting NMT protein substrates involves the systematic removal of amino acids from a high-affinity octapeptide NMT substrate that are not essential to the enzyme’s key recognition and tight-binding functionalities (23). This approach has resulted in peptidomimetic inhibitors exhibiting up to a 250-fold selectivity for the NMT of \textit{C. albicans} (a fungus accounting for serious systemic infections in immuno-compromised humans) over human NMT.

Antimyristoylation applications must also include strategies for avoiding general cellular toxicity. To date, the use of heteroatom–myristate analogues to modify the function(s) of specific \textit{N}-myristoylproteins appears to offer the best promise of accomplishing this goal. In the specific case in which a certain \textit{N}-myristoylprotein is rate limiting and required for proliferation of a particular cancer, then even a partial inhibition of global \textit{N}-myristoylation with relatively weak NMT inhibitors might reduce the steady-state concentration of that essential \textit{N}-myristoylprotein and selectively inhibit tumor growth. Similarly, in the event that the cancer-dependent (or viral-dependent (2)) \textit{N}-myristoylprotein is a catalytically inefficient substrate for NMT, then a partial inhibition of NMT may impede malignancy. Finally, a tumor that is dependent on the presence of unique \textit{N}-myristoylated proteins having individual half-lives significantly shorter than those of most other \textit{N}-myristoylproteins might be selectively killed by transient total inhibition of NMT (24).

Clearly, much more information on the role of \textit{N}-myristoylation in cancer and a clarification of the in vivo mechanism regulating protein \textit{N}-myristoylation in mammalian cells will be required to devise nontoxic \textit{N}-myristoylation-based chemotherapeutic strategies. The increase in NMT activity in colon cancer reported by Magnuson et al. (10) provides important new information for advancement toward this goal.

\section*{References}

Multidrug resistance in vitro: the multidrug resistance (MDR1; overexpression of a number of proteins has been associated with the ability of cells to efflux drugs as a result of decreased drug influx and altered expression of certain cellular proteins). Depending on the anticancer drug used for selection, multidrug resistance can be broadly categorized as 1) reduced accumulation of cytotoxic drugs and 2) the development of new therapies to circumvent multidrug resistance.

During the past two decades, two major directions of research have focused mainly on inhibiting expression and/or mutations in tubulin isoforms (14-16). In addition, multidrug resistance has been ascribed to reduced expression and altered activity of topoisomerase II (10-13) or altered expression and/or mutations in tubulin isoforms (14-16). Each of these proteins has been associated with a specific multidrug-resistant phenotype, and each may be viewed as a therapeutic target pending proof of clinical significance in drug-resistant human cancers.

Current strategies to circumvent multidrug resistance in the clinic have focused mainly on inhibiting expression and/or activity of the human MDR1 gene product, the best characterized mechanism for pleiotropic multidrug resistance. P-glycoprotein confers multidrug resistance by functioning as an efflux pump for a diverse assortment of anticancer drugs, including vinblastine, vincristine, doxorubicin, daunorubicin, pacitaxel (Taxol), etoposide, teniposide, and dactinomycin (2,17). There is now significant evidence supporting an association between P-glycoprotein expression in tumor specimens from patients and a poor outcome. See “Note” section following “References.”

Chemosensitizers to Overcome and Prevent Multidrug Resistance?

Ursula A. Germann, Matthew W. Harding*

The emergence of multidrug resistance is a well-recognized and major obstacle to successful chemotherapy of cancer. While chemotherapy is often quite effective in treating certain cancers, including Hodgkin’s disease, large-cell lymphoma, acute lymphocytic leukemia, or testicular cancer, most other types of cancer respond to chemotherapy poorly or partially (i.e., an initial response to chemotherapy followed by a relapse associated with resistance to anticancer drugs). In the laboratory, multidrug resistance has been modeled by selecting cultured cells in the presence of a single cytotoxic agent (e.g., doxorubicin), which usually leads to cross-resistance to a variety of anticancer drugs that differ in structure and mechanism of action (e.g., anthracyclines, vinca alkaloids, taxanes, and epipodophyllotoxins). During the past two decades, two major directions of research on multidrug resistance have emerged: 1) the elucidation of the genetic and biochemical bases for the multidrug-resistant phenotype and 2) the development of effective new therapies to circumvent multidrug resistance.

Several differences have been defined between multidrug-resistant cells and their drug-sensitive counterparts. They may be broadly categorized as 1) reduced accumulation of cytotoxic drugs as a result of decreased drug influx and/or increased drug efflux, 2) altered expression and/or activity of certain cellular proteins, and 3) physiologic changes that alter the intracellular milieu. Depending on the anticancer drug used for selection, overexpression of a number of proteins has been associated with multidrug resistance in vitro: the multidrug resistance (MDR1; also known as PGY1) gene product P-glycoprotein (1,2), the multidrug resistance-associated protein Mrp (3,4), and the lung resistance protein LRP (recently identified as the major vault protein) (5,6) as well as several enzymes (7-9). In addition, multidrug resistance has been ascribed to reduced expression and altered activity of topoisomerase II (10-13) or altered expression and/or mutations in tubulin isoforms (14-16). Each of these proteins has been associated with a specific multidrug-resistant phenotype, and each may be viewed as a therapeutic target pending proof of clinical significance in drug-resistant human cancers.

Current strategies to circumvent multidrug resistance in the clinic have focused mainly on inhibiting expression and/or activity of the human MDR1 gene product, the best characterized mechanism for pleiotropic multidrug resistance. P-glycoprotein confers multidrug resistance by functioning as an efflux pump for a diverse assortment of anticancer drugs, including vinblastine, vincristine, doxorubicin, daunorubicin, pacitaxel (Taxol), etoposide, teniposide, and dactinomycin (2,17). There is now significant evidence supporting an association between P-glycoprotein expression in tumor specimens from patients and a poor outcome.

*Affiliation of authors: Vertex Pharmaceuticals Incorporated, Cambridge, MA.

Correspondence to: Ursula A. Germann, Ph.D., Vertex Pharmaceuticals Incorporated, 40 Allston St., Cambridge, MA 02139-4211.

See “Note” section following “References.”